

REMARKS

Claim 1 has been amended to delete the penultimate clause, which the Office considers redundant. Upon reflection, the phrase “a second signal generating moiety which emits the same wavelength at the same intensity from all said different labels” is sufficient to describe the metes and bounds of this characteristic. With respect to the term “hue,” no amendment has been made for reasons that will be explained below.

In claim 3, the method outcome has been amended to conform to the preamble and the claim has been otherwise amended for clarity. The limitations of claim 9 have also been added to claim 3 as the purpose of the invention is to provide this method of measuring the number of particulate labels. A review of the amendment will show no new matter has been added and entry of the amendment is respectfully requested.

The Invention

The invention solves a problem inherent in the multiplexing capabilities of the invention labeling system. The problem is that the particles contained may be so small that the limits of resolution, which are imposed by the wavelength of detecting radiation, make it sometimes impossible to visualize individual particles, because the wavelength of the light is longer than the diameter of the particles. Thus, if the wavelength of light used for detection is, for example, 750 nm, resolution to levels smaller than 750 nm is impossible, *i.e.*, the detection space must be at least 750 nm. Thus, a multiplicity of for example 50 nm particles could be contained in this space. It will be important to count the number of particles that actually generate the signal observed from the space, both because this number gives an index of the dimensions of the targeted substance in

the detection space and because ambiguity is created by the manner of generating the characteristic hue associated with the particle.

As described in the specification in paragraph 22, the hue characteristic of a particle is the result of the ratio of more than one signal generating moiety, for example a fluorophore. To take the simplest possible example, if the hue generated by particle A results from equal ratio of red fluorescence to green fluorescence, the presence of one such particle would be indistinguishable from the presence of one red particle and one green particle, unless the number of particles emitting these fluorophores can be ascertained.

The invention provides for this by using a signal generating moiety that can be used as a particle counter. This signal generating moiety has a characteristic wavelength which differs from any of those used to generate the hue and is of constant intensity. Therefore, the intensity of the “counter” wavelength is a direct index of the number of particles being observed within the detection space.

When a composition such as that described in claim 1 is used to create a pattern, each detection space within the observed area can be evaluated for the type of substance present (by virtue of the hue of the particle(s) contained in that detection space), which will be unambiguous by virtue of the second signal generating “counter” moiety. To revert to the previous, perhaps oversimplified example, because of the “counter” it will be possible to distinguish between a single particle that emits both red and green from a combination of two particles, one of which emits red and the other green.

Formal Matters

Claims 1 and 2 were rejected under 35 U.S.C. § 112, second paragraph based on the recitation of the term “hue” as assertedly indefinite. Applicants appreciate the suggestion of the Office to substitute “wavelength,” but this appears inappropriate as the hue is generated from a combination of wavelengths. It is believed that one of ordinary skill reading only the claim would understand the meaning of “hue”; however, even if that were not the case, the nature of “hue” is clearly explained in the specification. As set forth on page 2, in the middle of paragraph 9, “the different characterizing light emitting moieties are generated by at least two signal generating moieties where each of the moieties generates a signal different from that generated by the other(s) and wherein the magnitude of such signals is varied among the different labels whereby each different label is thereby characterized by a different hue.” This is further described in paragraph 17. Thus, even if the skilled artisan were unclear as to the meaning of “hue,” the specification provides ample guidance. Applicants see no useful alternative to this term in light of the explicit explanation of its meaning in the specification and in view of the general situation wherein more than one wavelength is employed to provide the characterizing hue.

For further clarity, reference is made to the Nie publication (U.S. 2003/0148544), Figure 5, which substitutes the term “optical code” for “hue.” As shown in that figure, the ratios of the various wavelengths in the “optical code” create the hue that characterizes each particle. It will be noted, as further explained below, that the Nie particles fail to contain a “counter”; rather, they may be associated with analyte, which is itself labeled (not the particles themselves).

With respect to claim 3, the amendment is believed to obviate this rejection. With respect to the meaning of the detection space of 400-750 nm, assuming that wavelengths as long as 750 nm

must be measured, the detection space can be no smaller. If only wavelengths of 400 nm or shorter are used, the detection space can be no smaller than 400 nm across. This, too, is described in the specification in paragraph 13.

In light of the amendments to the claims and the foregoing discussion, it is believed that the rejection under 35 U.S.C. § 112, second paragraph may properly be withdrawn.

The Art Rejections

Claims 1-4, 7, and 9 were rejected under 35 U.S.C. § 102(e) as assertedly anticipated by Nie. Respectfully, it is believed that the disclosure of Nie has been misread.

According to the Office, Nie describes a composition comprising a multiplicity of different labels, each comprising a particulate support to which is coupled one or more first signal generating moieties that generate a visible hue that characterizes a label. So far, so good. The Office then asserts that each particle comprises a second signal generating moiety that emits the same wavelength at the same intensity from all said labels, citing “an all quantum dot system can be synthesized wherein a single QD is used as the analyte signal.” The quote is accurate, but the interpretation is not. It is the analyte, not the particle, that contains the second signal generating moiety. It is correct that the intensity of signal generated by the “tag” is in direct proportion to the amount of target present in the sample, but this is because the tag is attached to the analyte, not to the particle itself. Such an arrangement is clearly disadvantageous, since it requires pre-labeling of the analytes to be measured, whereas the method of the present invention does not.

In more detail, as explained above, the method of the invention permits the practitioner to determine the number of particles that are associated with a detection space in an environment

wherein the detection space is able to accommodate more than one particle. The system described by Nie is the opposite – it permits the assessment of the number of analyte molecules bound to a single particle. This is shown fairly clearly in Figure 5 of Nie which, for purposes of illustration, assumes that only a single analyte (shown as labeled with “F”) is bound to each particle. This would normally not be the case; a multiplicity of analyte molecules would be thus bound. This is spelled out in paragraph 75 of Nie. As indicated in the last sentence, the coding signals (*i.e.*, the hue of the relatively large particles which further contain a specific probe) identify the nature of the target (the DNA sequence), while the target signal (*i.e.*, the fluorescence emitter attached to the analyte, *i.e.*, target) indicates the presence *and the abundance* of that sequence.

Paragraph 76 describes the result of an actual assay (as opposed to the schematic in Figure 5). As described in paragraph 76, it was estimated that each bead contained no more than 24,000 probe molecules and no more than 10,000 target molecules. The results referred to in Figure 6 show that the target signal is in fact variable in any sample, because it is unlikely that the concentration of each individual target will be the same.

To put it more simply, the compositions and methods of the present invention may quantify and distinguish unlabeled analytes by counting how many particles are present on one analyte containing location (detection space) while Nie counts how many analyte molecules are on a single large particle.

Thus, Nie and the present invention are, in effect, opposite. While both employ multiplicities of beads, each with a biospecific binding reagent and a characteristic hue, the invention employs a “counter” signal attached to each particle so that the number of particles present in a detection space can be counted. Nie does not employ particles with a “counter” signal,

but rather places a counter signal on the target so that the number of target molecules associated with each particle employed by Nie can be counted.

It is therefore clear that Nie does not anticipate claim 1 or 2. Even more certainly, Nie does not anticipate claim 3. Nie does not generate a pattern of target substances, but rather provides a biological assay for the quantity of target substances in a sample. This is evident from both Figures 5 and 6. There is no spatial pattern described.

With respect to claim 4, the Office points to Figure 4A, assertedly indicating that the environment is displayed on a support suitable for viewing by microscopy. But Figure 4A is simply a display of the multicolor beads themselves, as set forth in Nie paragraph 21. It does not provide a display of an environment being assessed by the composition of beads. With respect to claim 7, this claim is not anticipated for all of the reasons set forth above. The inclusion of particles of 20-200 nm in the ranges set forth in Nie is clearly boilerplate, and it is clear from the same paragraph that the particles are preferably much larger – 500 nm-5 μ .

With respect to claim 9, the description by the Office is accurate, but supports the distinction set forth above. The reason that the signal generated by the tag attached to the target molecule is in direct proportion to the target present is that the tag is attached to the target in constant amount, not to the characterizing particulate in constant amount.

For these reasons, it is believed that the rejection over Nie may properly be withdrawn.

Claims 5-6 were rejected as obvious over the combination of Nie with Kauvar *et al.* (U.S. 2001/0044116). This rejection may be withdrawn for the same reasons set forth above; Nie simply does not teach the method of the invention, but rather its opposite.

CONCLUSION

The claims have been amended for clarification. The primary reference, Nie, fails to teach the composition of claim 1, as the particles in Nie's composition do not contain a second signal generating moiety that emits the same wavelength at the same intensity from all the different labels in the composition. Nie's compositions do not contain a second signaling moiety at all unless they are bound to a target which itself bears a second signaling moiety. Thus in Nie, unless all analytes are present in a sample at exactly the same concentration and the number of binding moieties on each of the particles is the same for each particle in the composition, the same "counter" signal intensity will not be characteristic of each particle in the composition. This is a vanishingly small possibility. In any case, Nie's "counter" signal is attached to the target, not the particle itself. Accordingly, Nie does not anticipate the present invention, nor does Nie in combination with Kauvar render claims 5 and 6 obvious.

Applicants respectfully request claims 1-8 be passed to issue. Should formal matters remain that might be resolved over the telephone, a call to the undersigned at the number below would be appreciated.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicants petition for any required relief including extensions of time and authorize the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket No. 388512010900.

Respectfully submitted,

Dated:

Jan 6, 2005

By:

Kate H. Murashige

Kate H. Murashige
Registration No. 29,959

Morrison & Foerster LLP
3811 Valley Centre Drive,
Suite 500
San Diego, California 92130-2332
Telephone: (858) 720-5112
Facsimile: (858) 720-5125